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Towards a sustainable pest management of the invasive bug *Halyomorpha halys* in Trentino province, Italy

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Abstract

Some invasive insects are agricultural pests, like the brown marmorated stinky bug (BMSB), an extremely phytophagous pentatomids. The lack of efficient low-impact control strategies makes this insect extremely dangerous for agricultural crops. In Italy, the first established populations of BMSB were found in 2012. In 2016 the insect appeared in Trentino, an intensive apple growing district.

In this Thesis, a mixed method study was conducted to explore vibrational communication of BMSB as well as to analyse the stakeholder's perception about the pest in Trentino.

The vibrational communication of BMSB was investigated by using a laser vibrometer, playback signals, a custom-made cardstock and wooden arenas. Results from the behavioural experiments with vibration showed that signal characteristics, such as frequency and temporal pattern, can trigger or inhibit male search. Natural and edited female vibrational signals were broadcasted on different settings. Using natural female signal, almost 50% of the adult males tested actively searched for the vibrational source, with 70% reaching it within few minutes after release. This opens the possibility to develop new trapping devices for monitoring and mass trapping BMSB.

Stakeholders' perceptions were collected through semi-structured interviews. Interview transcripts were analysed using thematic analysis (TA). Respondents had similar views on the possible different control strategies: whereas insect nets and insecticides were considered not feasible solutions for the area, biological control by the exotic parasitic wasp *Trissolcus japonicus* was considered as the most promising solution. However, according to the analysis conducted, the national department for agriculture did not give the permission to investigate the biological control efficacy and the possible unwanted effect on the local ecosystem by *T. japonicus* in Italy. The lack of this permission was identified by the stakeholders as a main institutional slowdown towards the development of sustainable BMSB control in Trentino.

Keywords: Hemiptera, invasive species, social survey, integrated pest management, biotremology.

Foreword

In the summer of 2018, I had the chance to meet many apple growers in the province of Trento. Some of them, were seriously concerned about “another alien pest” that was predicted to make apple growing even harder. By that time, in most areas the brown marmorated stink bug was not present and wasn’t for sure a pest for their crops. What captured my interest was that farmers were very eager of information and I couldn’t provide them any. I knew as much as them about it!

Then, during the autumn, I had the chance to meet my supervisor, Marco Tasin and we often discussed about the new challenges brought by this invasive pest. One day he informed me about the chance to join Valerio Mazzoni’s research group. At that time I had never heard about vibrational communication, anyways, I decided to try this new frontier of entomology.

During the social science courses of the Agroecology Master I learned how diverse the meaning of Agroecology can be. Some in fact perceive it as a new science, whereas many others, especially in developing countries, live it as an everyday political act. Personally, I really like the definition of agroecology as transdisciplinary, Participatory and Action oriented approach.

Soon I discovered that combining different scientific expertise is not an easy task. Soon, I started wondering how it was possible to use approaches such as Participatory Action Research in highly intensive and globalized apple districts such as Trentino. The desire to bring what I learned in Sweden, to my region in Italy was the force that allowed me to try this academic study in the frame of agroecology.

During the interviews for this thesis, I often happened to be asked by farmers: “why does a researcher ask questions to a farmer?”. When it happened I had a bitter-sweet feeling: on the one hand I felt not understood, on the other I realized there is lots of works for agroecologists.

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Abbreviations

APOT	Associazione Produttori Ortofrutticoli Trentini (Trentino Fruit Producers Association)
BMSB	Brown Marmorated Stink Bug
DFA	Department for Agriculture
FEM	Fondazione Edmund Mach
IPM	Integrated Pest Management
MD	Mating Disruption
MMX	Mixed Method Research
OPs	Organizzazione di Produttori (Producer organization)
PAR	Participatory Action Research
SSI	Semi Structured Interview
TA	Thematic Analysis

1. Introduction

1. Agroecology: an interdisciplinary approach

This thesis was written in the frame of agroecology, in the sense that it attempts to provide a systemic description of the emerging issues of a local bio-district, Trentino, including a set of laboratory experiments on novel pest control strategies.

In very broad terms, agroecology is defined as the ecology of agriculture. Francis *et al.* (2003), explain that despite a great ability in destroying natural ecosystems, human beings possess the great ability to design systems that are functional and thus resemble the characteristics of natural ecosystems. This is not an easy task, since agriculture can be defined as an “open system”, which interacts with natural flows and social forces. Considering this complexity, in order to analyse resilient agroecosystems, an interdisciplinary approach is essential to include all the dimensions. In other words, as suggested by Méndez *et al.* (2013), agroecologists have to simultaneously pursue to expand knowledge within specific fields while engaging with social sciences and broader agro-food systems.

One of the key principles of agroecology is the reduction of dependency on external inputs (FAO, 2016). Input reductions can be achieved in different ways, depending on the socio-economic context of a specific area. The present study focuses on pest control and on this topic, the most “mainstream” agroecological approach is the one proposed by Altieri and Letourneau, (1982), who suggest plant diversification to improve environmental performances for pest control. However, in highly specialized, market oriented growing districts, this may not be achieved within the short-term and thus other alternatives must be explored.

This study aims to explore new experimental approaches for pest control (Biotremology), and also to analyse the local context and its stakeholder's perception on pest control (Stakeholders analysis).

2. Theoretical framework of the study

This present work brings together two sub-researches: one of natural sciences and the other of social sciences. The problem with Mixed-Method Research (MMR), which uses both quantitative and qualitative methods, is that it involves the adoption of diverging epistemological paradigms. Indeed, biotremology was characterized by a post-positivist framework based on objectivism. The tests were carried out in a simplified laboratory environment. Careful observations were very important to test theories and the objectivity was important to avoid bias.

The stakeholder analysis was developed within a worldview of constructivism, which is a classic approach to qualitative research (Creswell, 2003). The study considered social reality as subjectively and culturally shaped. This means that reality was considered fluid and that every person holds his/her own reality, which was dependent on context, local conditions and personal experiences (Guba & Lincoln, 1994, as cited in Mühlhäuser, 2017, p.16). Since multiple realities were considered, the research focused on the complexity of views and also "to rely as much as possible on participant's views of the situation being studied" (Creswell, 2003). Finally, the aim was to interpret reality by actively engaging with participants and understanding the cultural context.

In order to avoid the frictions emerging by using two different epistemologies, with their opposed underlying assumptions about the essence of the reality (ontology) and its knowability, MMR adopts a pragmatist paradigm. As noted by Morgan (2014), pragmatism avoids "relying on metaphysical assumptions about ontology and epistemology" and it "recognizes the value of those different approaches as research communities that guide choices about how to conduct inquiry". In this sense, pragmatism treats differences in methods "as social contexts for inquiry as a form of social action, rather than as abstract philosophical systems". Following this framework, MMR rejects epistemological choices (Cameron 2011) and posits that "the use of quantitative and qualitative approaches in combination provides a better understanding of research problems than either approach alone" (Creswell and Plano Clark 2007).

Mixed methods are used in a practical and outcome-orientated way of inquiry that promotes “methodological appropriateness to enable researchers to increase their methodological flexibility and adaptability” (Cameron 2011). As Morgan put it, “for most of the researchers operating within the field of MMR, the appeal of pragmatism was more about its practicality than in its broader philosophical basis” (2014, p.1051). Instead, “pragmatism points to the importance of joining beliefs and actions in a process of inquiry that underlies any search for knowledge” as it treats methods and paradigms “as a set of beliefs and actions that were uniquely important within a given set of circumstances”. In this way, it is possible to combine different methods in order to gain a better understanding of phenomena that are oriented toward practical outcomes.

3. Insects and agriculture

Insects appeared 390 million years ago and diversified greatly into millions of species, which have adapted to almost all ecosystems on the planet. Due to their great adaptability, some insects compete with humankind for food resources by attacking cultivated crops (Vreysen, Robinson and Hendrichs, 2007). About one third of worldwide grown crops are lost, and insects have heavily contributed to such losses (Riegler (2018).

Projections in a global warming scenario, indicate that the highest levels of insect-caused crop losses will occur in temperate regions due to higher population growth, yet tropical regions are currently experiencing the highest damage from insects (Deutsch *et al.*, 2018). Moreover, mobility of people and goods has increased at an unprecedented pace over the last decades, leading to species redistribution that has major impacts on ecosystems and agriculture (FAO, 2001).

Pests have been generally controlled with the use of insecticides, especially after the green revolution. This increased crop yields and thus dropped food prices, especially in industrial countries. The application of insecticides have been controversial and very debated for decades. Insect suppression via chemical pesticides may indeed present a set of drawbacks, such as: lack of specificity towards target organisms, development of resistances and associated control failures, environmental pollution and deleterious effects on human health (Ansari *et al.*, 2014). For these reasons, more efforts should be done to develop biopesticides and alternative methods to manage pests, paying attention to promote favourable balances for biological control.

4. Integrated pest management (IPM)

IPM developed after the scientific community realized that single-method approaches failed to provide solutions to pest problems (Brader, 1979).

IPM controls pests utilizing regular monitoring to determine if and when treatments are needed and employing physical, mechanical, cultural and biological tactics to keep low enough populations to prevent intolerable damage. Least-toxic chemicals are used as a “last resort” (Olkowski, 1991).

In many growing districts world-wide, IPM boosted an agronomic change with diversified approaches to tackle pest outbreaks.

5. Apple IPM in the Trentino district

Trentino is an Italian alpine province, located in the North East of the country where almost 540.000 inhabitants live, mostly in the valley bottoms (ISTAT, 2016). It is a mountainous area, influenced by continental climate, with most of the territory lying 1000 meters above sea level and around 55% covered by coniferous and deciduous forests (Malek *et al.*, 2018). The climate at the lower altitudes is characterized by a sub-mediterranean influence.

Intensive agriculture is present close to the urban areas and the villages. The main crops grown in the region are:

- **apples**, mainly for fresh consumption grown on about 11000 hectares with an average production of 500.000 t/year (Montanaro, 2018). Apple industry contributes to about 35 % of the agricultural GDP of the province which in 2013 accounted for 206 million euros (PAT, 2013).
- **vine grapes**, which are grown on a surface of about 10300 hectares (Consorzio Vini Trentino, 2017) and generate almost 16 % of the agricultural GDP of the province;
- **Soft fruits**, grown in tunnels on a surface of 332 hectares for a total value of about 21 million euros. The most widely grown crops are strawberries which account for 70 % of the total production (Fedrigoni, 2016).
- **Vegetables and cereals**, grown on a total surface of about 650 hectares (Sassudelli *et al.*, , 2004).

From 2000, a new set of practices and concepts were introduced in apple production including: pest monitoring, damage thresholds, population forecast, biological and biotechnological control methods and data reporting about pesticide distribution

The most striking results were achieved with the introduction of the mating disruption (MD) technique. MD was introduced to tackle the problem of growing resistance of the Codling Moth (*Cydia pomonella*) (Lepidoptera: Tortricidae) to insecticides, particularly to IGRs (insects-growth-regulators) and also to reduce the conflicts between rural and urban populations related to the massive use of insecticides

By 2016, in the Trentino-South Tyrol region, the neighbouring province is accounted for as well, the overall apple growing area where this technique is deployed accounts for 22100 ha (Ioriatti and Lucchi, 2016). In order to make mating disruption effective, a combination of efforts have been put in place to overcome the challenges of farms fragmentation and the mountainous relief of the area.

At the beginning, the efficacy of mating disruption in comparison to standard pesticide was investigated. Meanwhile, monitor strategies for *Cydia pomonella* were introduced to establish action thresholds. Later, researchers, growers, advisors and cooperative leaders started cooperating to extend this low-impact approach on a wide area scale.

The great achievement of MD are described by Ioriatti *et al.*, (2011), which showed that, between 2001 and 2009, the combination of least toxic chemicals and MD contributed to a drop by 24% of the Environmental Impact Quotient (EIQ) of apple production in the region. For hemipteran phytoplasma-vectors (apple proliferation and flavescence dorée), control strategies based on insecticides are still mandatory, because MD is not available to control them. Among Hemiptera, a new invasive species, *Halyomorpha halys* is seriously endangering apple orchards and IPM practices in many fruit districts of the Northern Hemisphere, including the Trentino province.

6. The brown Marmorated Stink Bug (*Halyomorpha halys*)

1. Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is a phytophagous pentatomid species from Asia that is distributed in China, Japan, Korea and Taiwan (Zhu *et al.*, 2012). It has become a worldwide agricultural invasive pest that can feed on a wide variety of plants, both trees and herbaceous/ bushy plants). This pest is also well known among urban population due to its overwintering habits that makes it a major household nuisance.

This species, also known as brown marmorated stink bug (BMSB), was introduced from China in the USA in the late 1990s, from where it spread into Canada. In Europe, it was first found in Switzerland in 2007, and then in Italy in 2012. Later on, it was also in other European countries such as France, Germany, Serbia, Hungary and Rumania (Leskey and Nielsen, 2018). Re-

cently, it was found also in Chile (Faúndez and Rider, 2017).

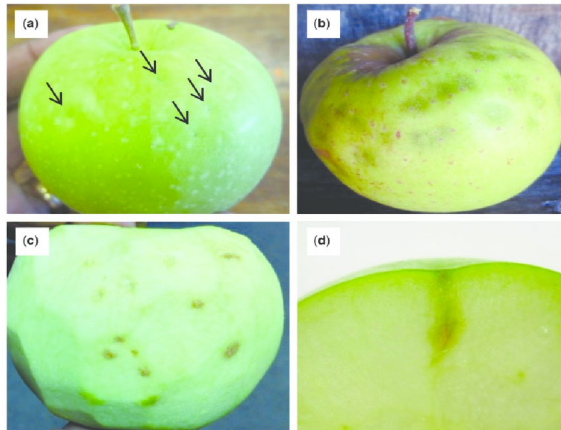


Figure 1 - BMSB feeding injury on apple fruit: (a) feeding puncture; (b) discolored depressions; (c) internal necrosis; and (d) stylet sheath (Shimat, 2015)

The BMSB feeds on a very wide host range including more than 170 species among which fruit crops, vegetables and ornamentals. The BMSB can move frequently from crops to wild vegetation and such movements are facilitated by its capacity to fly long distances (Lee and Leskey, 2015). Aggregatory

behaviour of this species can be particularly observed during the fall, on buildings where the adults find protected environments to overwinter (Lee *et al.*, 2014; Maistrello *et al.*, 2016).

Damage is caused by both nymphs and adults that while sucking on flower buds, fruits or stems, pierce and wound the surface to feed on the plants fluids, also injecting digestive enzymes (Haye *et al.*, 2014). Feeding injuries on fruits include “deformities, scars, discolorations and pitting that render them unmarketable”(Costi *et al.*, 2017).

2. Life cycle

The biology of *H. halys* has been investigated and reported for different areas in the world: China (Lee *et al.*, 2013), USA (Nielsen and Hamilton, 2009), Switzerland (Haye *et al.*, 2014) and Italy (Costi, Haye and Maistrello, 2017).

H. halys is a hemimetabolous insect and the time required for its full development from egg to adult spans from 40 to 60 days and depends on temperature and photoperiod. As reported by Penca and Hodges, (2019), eggs are laid on the lower sides of the leaf in clutches of generally 28 light blue eggs. *H. halys* is characterized by 5 nymphal instars. The first nymphal stage (Figure 2A) has a black head and orange red-abdomen, body size of around 2.4 mm. Successive molts progressively lose the red colour, turn dark grey,

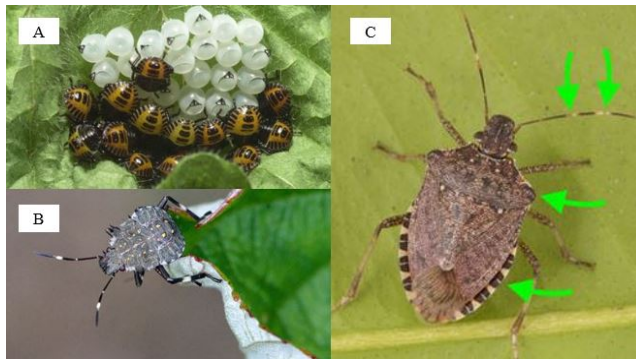


Figure 2 - Different morphological stages of *H. halys* taken from Penca and Hodges, (2019). (A) First nymphal stage, freshly hatched eggs (photo credit David R. Lance, USDA APHIS PPQ, Bugwood.org). (B) Late nymphal stage (photo credit Gary Bernon USDA APHIS PPQ, Bugwood.org). (C) Adult, green arrows indicate diagnostic features (photo credit Lyle J. Bass, University of Florida).

the thorax edges become spiny while from the third instar the wing buds become visible (Figure 2B). The body size of adults ranges between 12 and 17 mm in length, being the female larger than the male. Their body has a brown/red base color and black on the dorsal surface. Adults are characterized by the presence of four wings, which are not present in preimaginal stages. Key

features for identification of adults include white bands on legs and antennae and alternating dark and light bands on the abdomen (Figure 2C).

The number of generations per year greatly depends on the region. In China 1 to 2 generations have been reported for northern areas and up to 6 in the Guangdong area (Lee *et al.*, 2013). In New Jersey (USA), BMSB can complete up to 1 generation per year since eggs generally hatch in June and then go through their development while feeding on crops. Adults of the first generation start overwintering from September-October being active the

next year (Nielsen and Hamilton, 2009). In Europe, a single generation was observed by Haye *et al.*, (2014) in Switzerland. In Emilia-Romagna region of Italy, the insect completes two generations per year (Costi, Haye and Maistrello, 2017). Oviposition in Italy can occur at the beginning of May, allowing the insect to conclude a second generation which is also overwintering.

3. *H. halys* in Italy and Trentino

According to Cianferoni *et al.* (2018) the first BMSB in Italy was found in the region of Liguria, in 2007. However, the first established population report was done in the Province of Modena in 2012 (Maistrello *et al.*, 2016). After 2012, an important network of professional agronomists, researchers, students, entomologists and public press were alerted and invited to collect and record all findings of potential BMSB. Tracking BMSB was favoured by some initiatives of “citizen science” started in 2014, where, thanks to the support of professional entomologists, the presence of the species was mapped at an early stage (Figure 3).

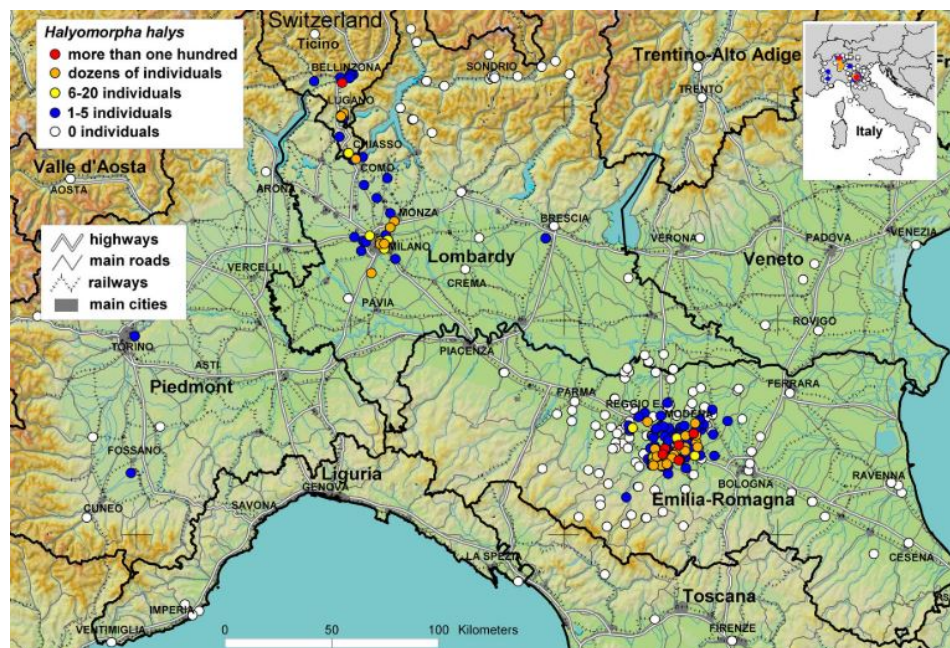


Figure 3 - Map of *H. halys* in Northern Italy and Southern Switzerland. Coloured circles represent the size of each finding (Maistrello *et al.*, 2016).

Within a short time, BMSB spread across the whole country. Despite climatic differences and the diverse agroecological landscapes the presence of BMSB spread not only in the Italian peninsula but, also to Sicily and Sardinia (Figure 4). So far, the northern regions are the most affected; Emilia-Romagna, Veneto and Friuli Venezia Giulia reported the highest damages on peaches, pears, apples and soybeans (Bianchi, 2018).

When BMSB was found for the first time in Trentino, in 2016 (FEM, 2016), the public extension service declared “a phytosanitary emergence” and started a monitoring plan. A smartphone application, was developed to track and report the presence of the insect and was later adopted from all the other Italian regions (Malek *et al.*, 2018).

In the last few years, national and local newspapers have been writing several articles highlighting the bad reputation of BMSB, defining it “a new enemy of fruits” (Trentino, 2018). Due to the relatively recent invasion of the species in the region, it is still unknown how much it will impact Trentino’s crops.



Figure 4- Year of first record of BMSB in Italy. Dark green = recorded before 2013; medium green = recorded in 2013–2014; light green = recorded in 2015–2017; white = no records (Cianferoni *et al.*, 2018).

Now, apples are the crops that could be most damaged by BMSB. As reported previously, this crop has a high economic and social relevance for the area. The local apple industry stakeholders including farmers, managers, researchers, farmer advisor and politicians seemed to raise different issues related to the invasive species. So far, no studies on stakeholders’ perceptions have been carried out but considering different views could promote the development of more holistic sustainable approaches.

7. Control strategies

When the population of BMSB grows and colonizes a specific district, the season-long application of chemical insecticides seems, at the moment, the only viable solutions for mitigating injuries on fruits and vegetables (Maistrello *et al.*, 2016; Short, Khrimian and Leskey, 2017). According to Leskey *et al.* (2012), in West Virginia, insecticide application frequency has considerably increased. Moreover, pyrethroids that were generally used to control native stink bugs, turned out to be inadequate to mitigate damages by BMSB. For this reason, growers started to rely on neonicotinoids, which were previously not used.

Insecticides registered for BMSB control have a broad spectrum and negative effect on beneficial insects (Roubos *et al.*, 2014). For this reason, biological control is currently one of the most investigated approaches to control BMSB. Hedstrom *et al.* (2017) performed some laboratory tests to assess the suitability of the samurai wasp *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) for BMSB biocontrol. The author tested no-choice and paired host-range tests, evaluating parasitism and host recognition on different pentatomids. Whilst no-choice tests showed similar host acceptance between BMSB and some other species, paired tests suggested a specificity of *T. japonicus* to BMSB eggs and therefore a great potential for BMSB biocontrol.

Another egg parasitoid of BMSB, *Anastatus bifasciatus* (Geoffroy) (Hymenoptera: Eupelmidae) was studied by Stahl *et al.*, (2019). The authors investigated the viability of mass release in an experimental orchard. According to the study, parasitism levels were not high enough to suppress the pest.

The predatory ability of ants belonging to the species *Crematogaster scutellaris*, was investigated on nymphal stages of BMSB. Castracani *et al.*, (2017) found no active predatory behaviour, but rather a predation that occurs by random “encounters”.



Figure 5 - Insect screen (Photo by Claudio Corradini)

Insect screens (Figure 5) seem to be the only method with high efficacy. The drawbacks of this control strategy are the high investment cost and the use of plastic. Italy's most affected region, Emilia-Romagna started subsidizing this method (Corradi, 2017).

In order to rationally choose control options, monitoring tools are very important. Researches still strive to develop and refine monitoring tools that can deliver information on the density of the population at different times.

Weber *et al.*, (2014) identified a three-component blend of volatiles as an aggregation pheromone for young BMSB instars and adults. The efficacy of this blend was higher in the late growing season, just before overwintering. The effectiveness of the trapping tools seems to depend on several factors, such as the design of the trap, the placement in the field and the architecture of the crop. According to Morrison *et al.*, (2015) economically viable tools for monitoring the population seem to be forthcoming in the near future.

Polajnar *et al.*, (2016) report a different view on pheromone traps developed for "attract and kill" strategies to control Pentatomidae, which tend to linger in the vicinity of the traps but rarely enter. This could be explained by the fact that, in Pentatomidae, aggregation pheromones are used as a generic attractant rather than a precise source of localization (Aldrich, 1988), while more accuracy should be given by sexual stimulation which in Hemiptera is generally provided by vibrational signals (Čokl and Virant-Doberlet, 2003).

1. BMSB vibrational communication

Animal communication is very fascinating and diverse. As a general scheme, an encoded content is sent and a receiver must detect and decode it. The signals used to communicate can be of different nature: visual, chemical and physical.

Physical communication occurs through acoustic and vibrational waves (Figure 6). Substrate-borne-vibrational communication is very ancient among arthropods and used by around 200.000 different species of insects on the planet. Biotremology is the discipline that studies substrate-borne vibrational communication.

Vibrational signals can be transmitted in different media present on the Earth (solid, liquid or gas) and their characteristics influence the transmission of the signal (Hill and Wessel, 2016). Signals are in general characterized by specific frequencies, temporal patterns and amplitudes. The right frequency can improve the signal transmission on the substrate and the temporal pattern (rhythm and repetition time) conveys information about the fitness of the insect. In contrast, the role of the amplitude is still debated (Cocroft *et al.*, 2014).

The continuity of the substrate allows the signal to create an active space for communication that in most cases coincide with host plants. Due to their great diversity, plants have very different physical properties which directly influence the transmission of signals. Under this conditions, the amplitude of the signals plays a very important role, providing information about directionality and distance (Cocroft *et al.*, 2014).

The communication channel of substrate-borne vibrational communication are boundary waves. Boundary waves can be either Rayleigh waves or bending waves.

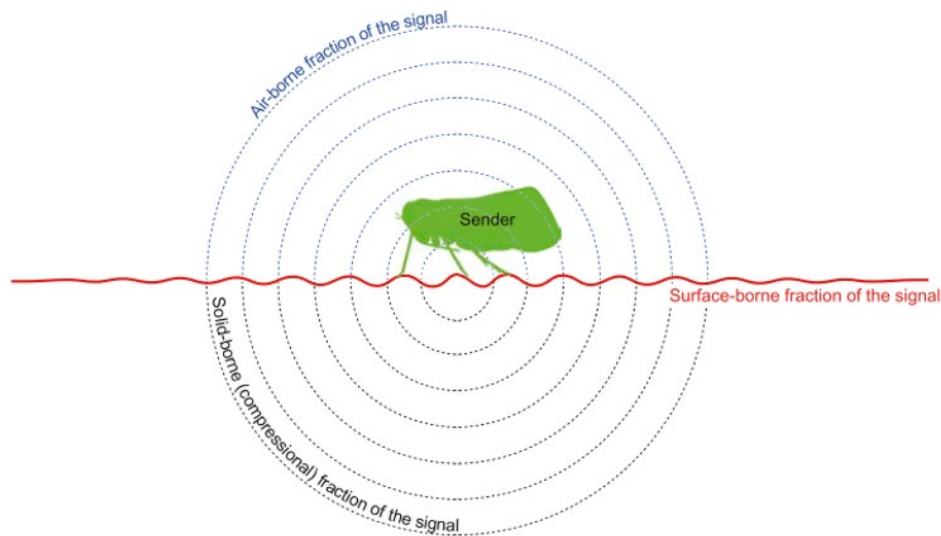


Figure 6 – Mechanical wave forms produced by a planthopper: air-borne and substrate borne fractions of the signal (Image taken from Hill and Wessel, (2016)).

The vibrational communication is highly variable in terms of content and signals can elicit behaviours that ranges from foraging or mating, to parental care (Hill and Wessel, 2016). Individuals who receive communicational signals have to obtain following information: who, what and where?

In their review Čokl and Virant-Doberlet, (2003), focus on *Nezara viridula* (Heteroptera : Pentatomidae) as a reference species for Pentatomidae. In this species, substrate-borne signals are produced through stridulation and vibration of abdominal tergites. In Pentatomidae, vibrational signals are perceived by sensory organs called subgenual and chordotonal organ. They are located in the legs and are characterized by the presence of mechanoreceptors.

Signals, also defined as songs, are characterized by low frequency peaks, around 100 Hz and are species specific. Song characteristics are well tuned to the transmission properties of plants. The amplitude of the signal depends on the characteristics of the plant substrate and its attenuation is stronger on soft and flexible stems.

In BMSB, mating behaviour is regulated at long distance by male-emitted aggregation pheromones. The aggregation pheromone is used in many

species of the family Pentatomidae to find a potential partner in the environment (Khrimian *et al.*, 2014). On the other hand, once an individual has landed in vicinity of another one, the mating is mediated by substrate-borne vibrations, as it is for many other insect orders.

Polajnar *et al.*, (2016) found that pre-mating behaviour of BMSB, follows a similar pattern as the one observed in *N. viridula*. When males land on a surface, they start emitting mating songs. If the female is willing to mate, it responds with a female call. The male responds and this behaviour is defined as a duet. When this happens usually the male starts actively searching (a typical walk and stop characterized by pushing up with the legs). The study showed that all mating pairs did not mate before the exchange of vibrational cues, even if insects found each other by chance.

Polajnar *et al.*, (2016) explained that male songs are characterized by long pulses with peak frequency at 50 Hz, downward frequency modulation and gradually increasing amplitude. the basic signal of the female is a single pulse with similar spectral structure of the male signal but three times shorter in duration. The authors found out that male and female songs change their characteristics according to the mating step. The article concludes with the observation that the male signals recorded didn't show attractiveness towards males or females, whereas female song (FS2) was highly attractive to males.

The study conducted by Mazzoni *et al.*, (2017), aimed to assess the attractiveness of the signal FS2 in different settings such as leaves and plastic. that the FS2 signal is attractive when broadcasted with mini-shakers and that the signal induces a relevant loitering effect (insects keep searching in the close vicinity of the stimulated area). The authors observed impaired orientation of the insects on large 2D surfaces. As explained in the article, this was attributed to the lack of adaptation of pentatomids to locate vibrational sources on such large flat surfaces. Despite not finding the source males didn't give up the search, indicating high motivation.

One of the tests conducted, tried to assess the trapping potential of the signal with a trap prototype and 50% of the males released were captured. As mentioned previously, commercial traps use the aggregation pheromone dispensers to trap BMSB. The use of vibrational signals combined with odour baited traps could provide important synergistic effect, improving the capture rate. More research is needed to define which signal characteristics can further improve it efficacy, especially in terms of spectral and temporal parameters (Mazzoni *et al.*, (2017).

2. Research questions

1. Biotremology

Currently, the research in biotremology focuses on understanding vibrational communication of BMSB, with the aim to develop monitoring and mass trapping tools. The biotremology experiments carried out during the lab experience had the aim to answer to the following research questions:

- *What are the parameters of the female call that are important to develop a highly effective and attractive signal to be employed for mass trapping and monitoring system for the BMSB?*
- *To identify the parameter values that best condition the BMSB behaviour, it is important to have an arena that can provide such information. At the moment, such device is not available. Is it possible to ideate a tailored arena and a protocol, for conducting 1 and 2-choice experiments?*

2. Stakeholder analysis

Whereas many scientific studies were published about the threat represented by BMSB to crops, to the best of our knowledge, no studies have been carried out about the perception of BMSB in a local context.

In order to gather the perception of the apple industry stakeholders about BMSB, a social survey was carried out on a sample of stakeholders. The research question was:

- *In the recently colonized Trentino province, what are the BMSB related issues perceived by the apple industry stakeholders?*

3. Materials and methods

Biotremology

1. Insects rearing

BMSBs adults were collected during autumn 2018 in Codroipo, Friuli-Venezia Giulia (Italy). During the winter, insects were placed in wooden boxes inside a hoophouse at FEM (San Michele a/A, Italy). Starting from February 2019, overwintering insects were reared on a mixed diet with fresh beans, tomatoes, kiwis, dried almonds, sunflowers and apples. Water was supplied ad libitum through soaked cotton. Rearing containers were cleaned, and food and water changed twice per week. Individuals were tested once or twice. When tested twice, it was not during the same day. Only adult males were used for experiments.

2. Bioacoustics laboratory

The experiments were conducted in the Bioacoustics laboratory of FEM (Trentino, Italy) on an anti-vibration table (Astel, Ivrea, Italy). Insects were tested within an acoustically insulated chamber (Trebi s.r.l., Trento) kept at 23 ± 1 °C in artificial lighting condition (50 lx). Tests were carried out between 2 and 6 pm.

3. Signal playback

During the experiments, 4 different playbacks of the female signal (pbFS) were tested. A detailed characterization of the signal is provided in table 1.

Table 1 - Characteristics of female signals used in the experiments. Temporal characteristics (duration of the signal, pauses and number of repetitions) and spectral features (peak frequency and intensity) of the signals are reported.

Playback	Duration	Repetitions	Pulse duration		Pause duration		Peak frequency					
			time [s]		time [s]		I Harmonic [Hz]	st.dev	Intensity [dB]	II harmonic [Hz]	st.dev	Intensity [dB]
	[s]	[n]	time [s]	st.dev	time [s]	st.dev						
80	11,4	12	0,58	0,07	0,39	0,09	73,15	2,34	139,93	163,67	2,05	121,92
160	13,4	12	0,66	0,06	0,46	0,09	74,30	2,46	107,82	165,78	2,50	129,06
even	13,3	12	0,71	0,06	0,42	0,07	71,33	1,76	123,47	163,23	2,07	124,91
slow	16,1	12	0,55	0,07	0,85	0,09	73,15	2,34	139,93	163,67	2,05	121,92

pbFS-80 was recorded from a BMSB female during a study conducted by Polajnar *et al.*, (2016) and corresponds to a natural calling signal, without any modification, and is characterized by a series of pulses at rather regular repetition time (i.e. interval between consecutive pulses, which is a parameter of “speed” of signal emission) and harmonic structure (Figure 7). The first harmonic (fundamental) is at 73 Hz and is dominant compared to the others, with a difference of almost 20 dB with the second harmonic at 164 Hz.

The other signals, shown in the Table 1, have been edited with the software Audacity 2.1.2, with the aim to evaluate the importance of these parameters (pulse repetition time and harmonics pattern) in eliciting attraction toward males. For this reason we questioned if (1) the rate of intensity between 1st and 2nd harmonics and (2) the speed of the signal emission influenced or not the behavioural responses of males. In this way we synthesized two pbFS: *pbFS-160* with inverted frequency pattern through intensity reduction of the 1st harmonic (-30 dB) and the amplification (+ 10 dB) of the 2nd one; *pbFS-even* is instead characterized by two harmonics at an equal amplitude of 124 dB. *pbFS80-slow* has the same frequency pattern as *pbFS-80* but a longer pause between pulses and thus a slower speed of signal emissions. Signals were transmitted to the experimental set-ups using mini shakers.

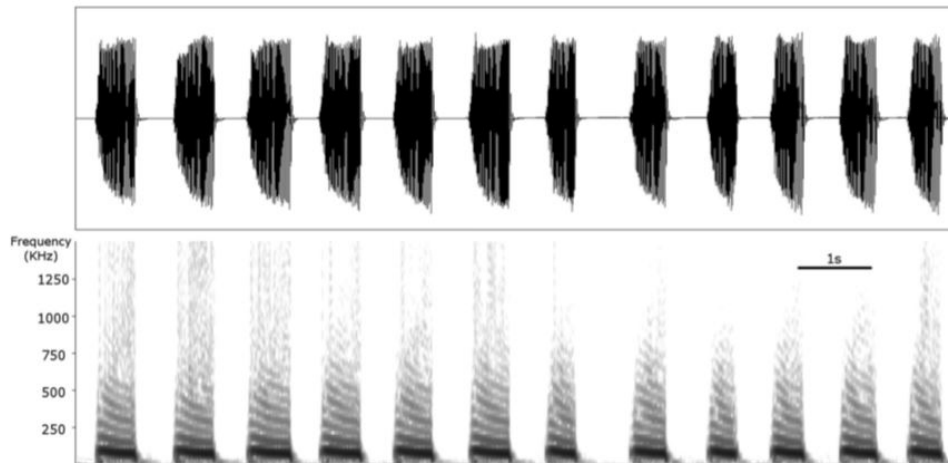


Figure 7 - Signal pbFS80 characterization, oscillogram (above) and (spectrogram) below taken from Mazzoni et al., (2017)

The signal was constantly monitored with a laser vibrometer (Ometron© VQ-500-D-V; Brüel and Kjær Sound & Vibration, Nærum, Denmark, Nærum, Denmark) targeting a small square piece of reflective sticker placed on the experimental arenas (see below). The monitored signal was digitized at a 48-kHz sample rate and 16-bit resolution. It was stored onto a hard drive through the LAN XI data acquisition device (Brüel and Kjær). The spectral analysis was performed with the software PULSE 14.0 (Brüel and Kjær). This setup was used for describing the vibrational amplitude of the arenas.

4. Tests

Two different experimental setups were tested, i.e. (1) a *custom-made cardstock arena* and (2) a *custom-made wooden T shaped arena*.

The (1) cardstock arena aimed to assess which playback encourages the male search the most, whereas the (2) wooden T-arena aimed to assess the ability to locate the shaking source.

1. Custom-made cardstock arena

The arena consisted of a circular base ($\varnothing = 30$ cm) surrounded with an “arena wall” made of 5 cm high cardboard strip. Two prong clamps were used to keep the arena suspended over the table. The vibration reached

the arena by way of a rectangular cardboard (4 x 22 cm) which balanced with one side on a wax (Surgident Periphery Wax, Australia) covered conical rod screwed on the top of a mini shaker (type 4810, Brüel and Kjaer) and the other laid in direct contact with the top surface of the cardboard arena (Fig. 8).

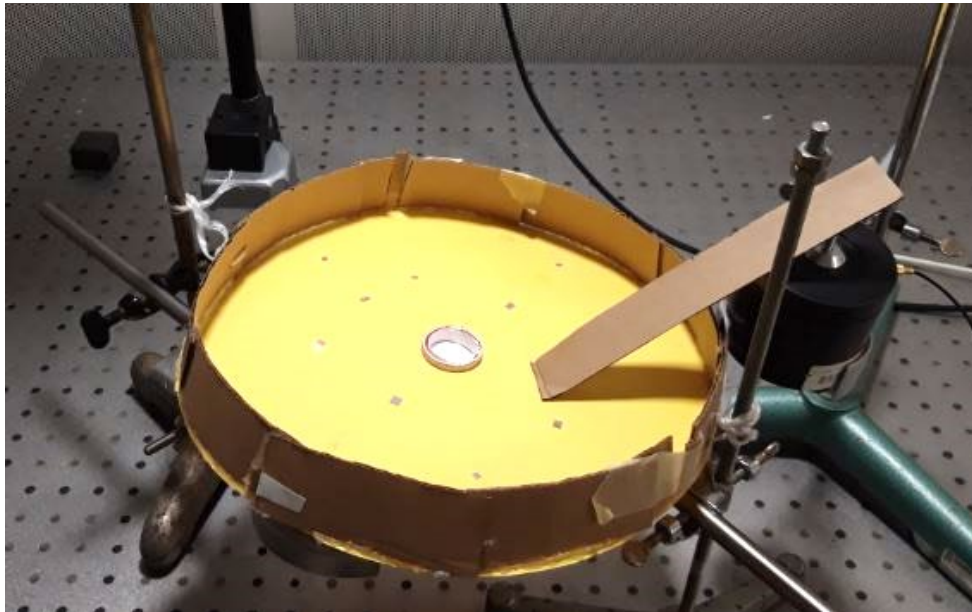


Figure 8 - Cardstock arena was used to conduct test 1 and 2. The rectangular cardboard touched on both the mini-shaker and the yellow base of the arena sending out the broadcasted signal. The insects were released in the white cap placed in the centre of the circle.

The insect release point (RP) was set in the middle of the arena where a Falcon vial cap ($\varnothing = 3.5$ cm, 1 cm depth) was built into the cardboard surface. The vibrational amplitude field was monitored from one audio sampling point (ASP) located on the cardstock arena, 5 cm from the RP.

Two tests were carried out with this experimental set-up:

- Test 1 - Dominance of the harmonics (pbFS80, pbFS160, pbFSeven)
- Test 2 - Pulse repetition time (pbFS80, pbFS80-slow)

For both tests, we counted the number of males that showed the searching behaviour when subjected to different playbacks.

In **test 1**, the playbacks were looped and played according to the protocol summarized in table 2.

Table 2 – Protocol test 1. A description of each step followed for the test.

Step	Description	Duration (min)
1	Place insect in the RP, covered with a cap	0
3	1 st Playback (randomised FS-80/FS-160/FS-even)	1
4	Pause	0.5
5	2 nd Playback randomised FS-80/FS-160/FS-even, excluded the one broadcasted as 1 st Playback	1
6	Pause	0.5
7	3 rd Playback (the one left, not yet broadcasted, among FS-80/FS-160/FS-even)	1

In **test 2**, the playbacks were looped according to the protocol summarized in table 3.

Table 3 - Protocol test 2, A description of each step followed for the test.

Step	Description	Duration (min)
1	Place insect in the RP, covered with a cap	/
2	Acclimation (before removing the cap)	0.5
3	1 st Playback (randomised FS-80 and FS80-slow)	1
4	Pause	0.5
5	2 nd playback (the one between FS-80 and FS80-slow not yet broadcasted)	1

2. Wooden custom-made T

The wooden T-arena consisted of 3 mm thick plywood and was built to mimic an “olfactometer”, for the one- and two- choice tests for insects stimulated with substrate-borne vibrations. The RP was set at the base of the T and two mini-shakers were applied at the lateral ends of each T arm (Figure 9).

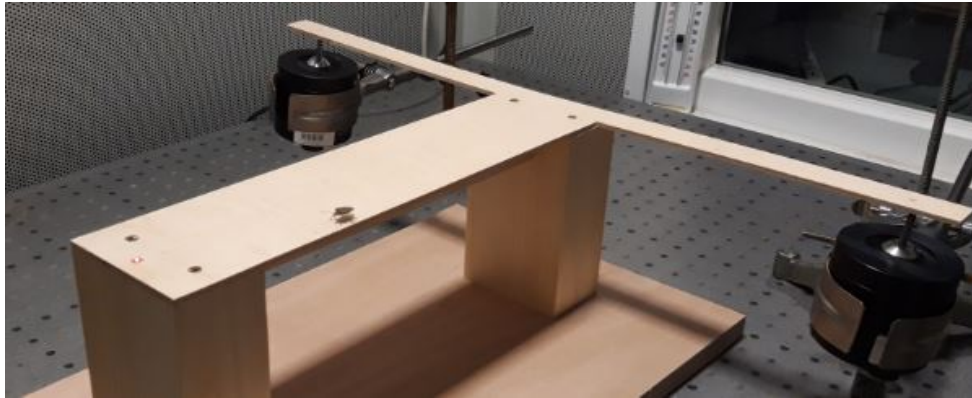


Figure 9 - Wooden custom-made T shaped arena. The insects were released in the “bottom part” of the T (left side in the picture), whereas at the two opposite extreme ends of the T, two shakers were placed to broadcast the playbacks.

To avoid any possible transmission of vibrations through the table, the mini-shakers were separated from it by prong clamps.

Before starting the tests, the intensity of the playbacks was mapped over different parts of the arena, to characterize the vibrational pattern of the arena and to ascertain the occurrence of a gradient directed to the working shaker, when only one shaker was active. In total 23 points were measured with laser vibrometer from the T-arena (4 on each arm and 15, divided in 3 rows, on the main stem) during the transmission of any of the playbacks. The final value of intensity (measured as velocity of substrate vibration, $\mu\text{m/s}$) was calculated as an average of 3 randomly chosen pulses (Figure 10).

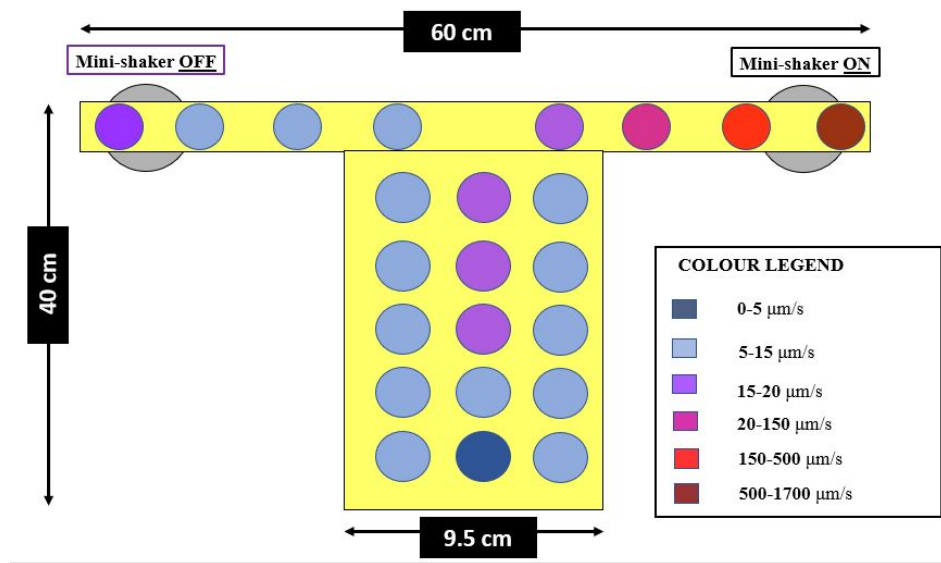


Figure 10 - Representation of the gradient intensity of the vibrational signal broadcasted. Intensity is expressed as substrate vibration velocity ($\mu\text{m/s}$) of the plywood.

On this experimental set-up, two tests were carried out:

- **Test 3** – One-choice test
- **Test 4** – Two-choices test

These tests aimed to observe the behaviour of the males released and assess their accuracy in reaching the shakers.

The playback used in **test 3** was *pbFS-80*. It was played according to the protocol shown in Table 5. Once the signal was on, it was continuously played until the end of the experiment. During the experiment, only one of the two shakers was on and the other was kept in the set-up to avoid visual bias. The working shaker was alternated after every release. Only active (i.e. searching) males were considered valid for the analysis. We took account of the number of individuals that reached the vibrating shaker.

The playbacks used in **test 4** were *pbFS-80* and *pbFS-160*. The protocol followed during the experiment is summarized in Table 5. The playbacks were played simultaneously (on different arms) to assess the capacity of the insects to distinguish between the two signals (see results, **test 1**). Our hypothesis was that if males could accurately distinguish between two qualitatively different signals, they would have chosen more often the mini-shaker

transmitting *pbFS-80*. The transmission of the two playbacks was inverted (between the two mini-shakers) between each trial.

5. Data analysis

Males were labelled as “active” when they showed “searching behaviour”, which was characterized as the characteristic walk and stop followed by some “push-ups” or “leg stretches” (Polajnar et al., 2016).

Data were collected by visual observation. In addition, for test 3 and 4, insects have been video recorded to double check the validity of the data collected. The experiment was stopped when the shaker was not reached within the maximum given time (see Table 4 and 5) and the test was considered finished when insects reached one of the tips of the two shakers. The time required to reach the shakers has also been registered.

The number of responding insects was submitted to a Yates’ corrected Chi-squared test. When the number of tested specimen was below 30, a Fischer Exact test was used. In test 1, to separate the effect of different treatments, a Ryan multi comparison test for proportions was used (Ryan, 1960).

Test 1, dominance of the harmonics – A total of, 103 males were released. We counted the number of searching males in correspondence of each playback and evaluated also a possible “position effect”, that can be defined as the influence of the position of each playback within the random sequence of the three transmitted playbacks. This was done, because we hypothesized a positive/negative influence of a playback to the following ones. In practice, a “very” attractive signal could potentially trigger higher response in a “poorly” attractive signal coming immediately after, and viceversa. In this way, we also compared the number of searching individuals before and after the transmission of any of the playbacks. In case of absence of “position effect”, no significant difference (after Exact Fisher’s test) would have been found between them.

Test 2, pulse repetition time – 25 males were released. A Chi squared test in contingency table (2x2) was used to compare the number of males that showed searching behaviour during the stimulation with *pbFS-80* and *pbFS-80-slow*. Again, the “position effect” was tested with the Fisher’s Exact test between positive (active) and negative (not active) responses before and after any signal.

Test 3, one choice test – 63 males were released. A chi-squared likelihood table was used to assess a significant accuracy of active males on the T-arena, by comparing the number of males that reached the working mini-shaker with those that did not. The time required to reach the target was also measured.

Test 4, two choices test – 40 males were released. A chi-squared likelihood table test was used to assess a significant preference between pbFS-80 and pbFS-160 on the T-arena.

Stakeholder analysis

Whereas many scientific studies were published about the threat represented by BMSB to crops, to the best of our knowledge, no studies have been carried out about the social dimension of the perception of BMSB in a local context. Méndez et al. (2013) explain that in western society approaches to sustainability and agroecology tend to obscure the relevance of social contexts in the process of knowledge creation. This post-positivist worldview (Creswell, 2003) has certainly played a very important role in the technical and biophysical achievements in the agricultural sector, which contributed to prosperity in western society. However, many social and cultural issues are mostly left unexamined and their potential in triggering changes is largely underestimated. In the area examined, it is rather uncommon to have participatory groups where views on specific issues are presented and discussed. Decisions related to agricultural strategies are mainly taken with a top-down approach.

In order to engage with the diversity of the apple industry stakeholders and include their perception, a social survey was conducted. The perceptions reported by the interviewees were used for the identification of common concerns and to identify possible solutions for the BMSB “phytosanitary emergency” (FEM, 2016).

6. Apple industry in Trentino: the institutional network

Apple represents the most widely grown crop in Trentino. In this growing region, the average surface of farms is 3 hectares. This relatively small

farms are often divided into several allotments (Sassudelli et al., 2004). Considering the alpine location of the area, orchards are also in general quite steep and difficult to mechanize.

Beyond single farms, Trentino's apple industry is composed by a set of interconnected institutions:

- OPs, Organizations of Producers – which are groups of cooperatives. Currently there are three OPS in the region: Melinda, La Trentina and Società Frutticoltori Trento (SFT). These three OPs together represent 95% of the producers in the province. Each OP is composed of several cooperative members and cooperatives own one or several storage plants for apple conservation and commercialization. The members of these three OPs are about 7500 (Trentino Agricoltura, 2018).
- APOT, Trentino Fruit Producers Association - an association which is in charge of: setting common IPM standards, monitoring the activity of the different OPs and discussing sanctions against OPs in case of noncompliance of common rules (APOT, 2018).
- Department for Agriculture (DFA) – it is charge of the actualization of European guidelines with regards to phytosanitary issues and agricultural legislation in general (PAT, 2018).
- FEM, Fondazione Edmund Mach – a research institute that carries out education, research and extension service in agriculture (FEM, 2018).

The rentability of farms along with their economic sustainability largely depends on cooperatives and OPs. The cooperation allows to decrease storage costs and to commercialize fruits in a more efficient way, even if the competition with other producing areas appeared to be tougher in the last years (Montanaro, 2018). In this system APOT has the role to develop partnerships with FEM, which conducts research in several areas of expertise.

A capillary extension service is provided by the different OPs to their members, in most cases through direct funding to FEM. A group of advisors for organic agriculture was created to support the growing number of producers that are growing organic apples. Advisors are responsible for monitoring activities to support IPM and organic practices.

7. Sample interviewed

Between March and June 2019, eleven stakeholders were interviewed:

- Farmers (N=6) - Respondent growers were all member of the OP “La Trentina”. Their age was between 25 and 50 and they had different degree of experience in fruit production. Growers were contacted either because they reported pest presence in their orchards or interest to talk about the issue. Half of them were organic producers, while all of them were professional full-time growers.
- Managers (N=3) – Two of the respondent managers were “head of production” for the OPs “La Trentina” and “Melinda”, with task such as the quality check of production from the field to the retailer. One was the direction of APOT.
- Researcher – The interviewed researcher had several collaboration projects with FEM and in the last years he focused on investigating BMSB from the first established population in Emilia Romagna. The research focus of her projects was about BMSB biology and control. She also took part to the organization of “citizen science” initiatives in Italy.
- Advisor – The farmer advisor worked for FEM towards organic farmers. Her area of expertise was on entomology and fungal diseases.
- Councillor for agriculture -The councillor was interviewed as head representative of the Trentino DFA.

As potential stakeholders, also citizens were taken into account in the preliminary phase. However, considering that the main interest of the study was about attitudes towards the insect control, this group was not included in the analysis.

8. Semi-structured interview (SSI)

Considering the relatively “new” area of the research (analysis on stakeholder’s perceptions are not common practice in agricultural research in Trentino), the method for data collection was a set of semi-structured interviews. According to guidelines provided by Adams (2018), interviews were conducted conversationally with one respondent at the time, blending open and closed-ended questions often followed by follow-up *how* or *why* questions. The sequence of stakeholders interviewed was mixed, not following a specific order. In most cases the dialogue delved into completely unforeseen issues. The length of the interviews lasted from 20 to 45 minute and were

recorded with a smartphone and transcribed using the software oTranscribe. Respondents were always asked to sign a permission document before the beginning of the interview.

Before starting the interviews, in order to build a stakeholder's network and familiarize with the main issues, a first "pilot interview" was conducted. During the pilot interview, an organic apple producer, who was also a member of the decisional council of an OP called "La Trentina", was interviewed. This preliminary phase allowed building an "interview guide". The interview guide contained the main relevant issue on the topic. This first meeting allowed obtaining the contacts to farmers, managers and advisor.

9. Analytic method

Thematic analysis (TA) was the analytic method used to interpret the information gathered from the interview transcripts.

TA is becoming widely used as qualitative analytic methods since it offers an accessible and flexible approach to analyse qualitative data also for those who are not particularly familiar with qualitative research. An exhaustive description and guidelines for TA is provided by Braun and Clarke (2008). According to the authors, this analytic method is essentially independent of theory and epistemology, in the sense the analysis is not guided by any underlying theory used to interpret data and reality in general. This allow a high degree of freedom to collect and interpret data.

The *data set* was represented by the transcripts of 11 SSI. The main focus here was, for each stakeholder group, to create a description of the issues perceived. Meaningful sentences, paragraphs and sometimes words were labelled with commonly recurring themes. Themes were considered relevant when connected to the research questions listed in Chapter 2. It is important to mention that in TA, the role of the researcher is essential in deciding what are themes of importance and thus to report them to the readers. In TA, themes and meanings do not reside in data but in the researcher thinking about the data. Braun and Clarke (2008) explain that themes are meant as representations of some level of "patterned response" within the interview transcripts. In this case, the focus is on providing the main issues that make the results from SSI easily accessible.

The TA involves "a progression from the description, where data are organized to show the patterns in semantic content, to interpretation, where there is an attempt to theorize the significance of the patterns and their broader

meanings and implication (Patton, 1990 as cited in Braun and Clarke, (2008))”.

4. Results

1. Biotremology

1. Cardstock arena - Test 1: “dominance of the harmonics”

In test 1, 20 out of 103 males (19 %) were active. Among the stimuli, the highest activation (17 insects) was achieved with the signal *pbFS-80*, while lower activation were observed with *the pbFS-even* (11 insects) or by the *pbFS-160* (9 insects; Figure 11) (Chi squared test: χ^2 : 7.3, df = 2, p = 0.026).

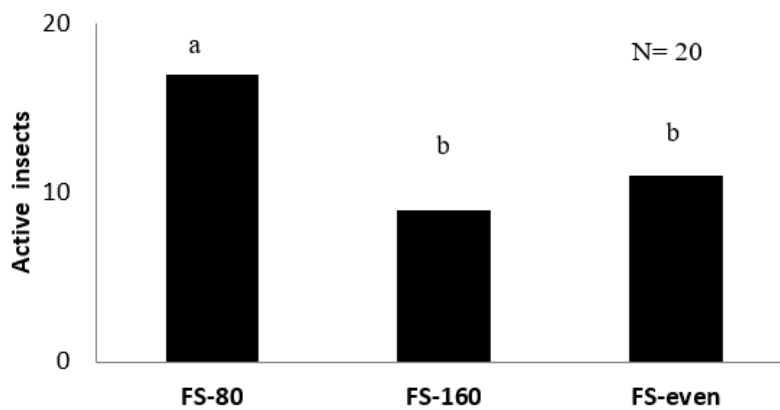


Figure 11 – Dominance of the harmonics. Number of insects activated by different playbacks. Different letters indicate significant difference between treatments (the playbacks) after χ^2 test in contingency table (2x3) followed by Ryan's test for proportions.

The analysis on the position effect of the playbacks (Figure 12), showed that the number of active insects was significantly higher after *pbFS-80* was played (Fisher's Exact test: $p=0.0035$). In contrast, the number of active insects decreased significantly ($p=0.0061$) after that signal *pbFS-even* was played. No effect was measured after the transmission of *pbFS-160* ($p > 0.05$).

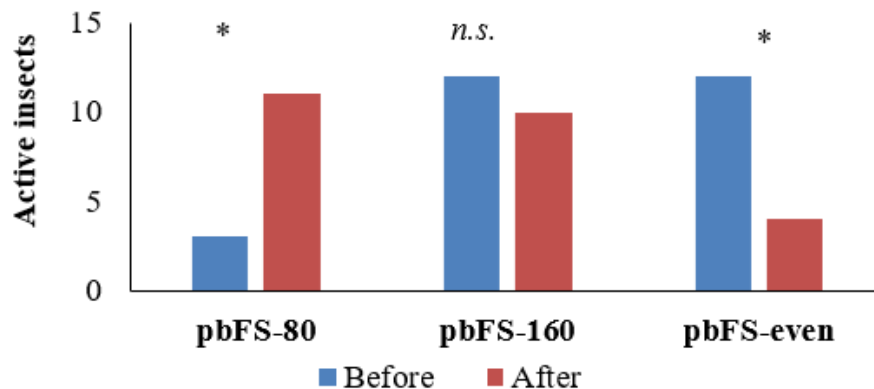


Figure 12 - Position effect of the playback, test 1. Number of insects active before and after any of the playback was played. Asterisks indicate significant difference ($p < 0.05$) Fisher's Exact test.

2. Cardstock arena - Test 2: "Pulse repetition time"

In test 2, 20 (out of 25) insects were active (80%) with a considerable difference with respect to test 1. Such difference might have been caused by the change in photo-period, but was not further investigated. The signal *pbFS80* activated more insects than the *pbFS80-slow* (χ^2 test in contingency table (2x2): $\chi^2 = 10.8$, $df = 1$, $p=0.00026$ (Figure 13).

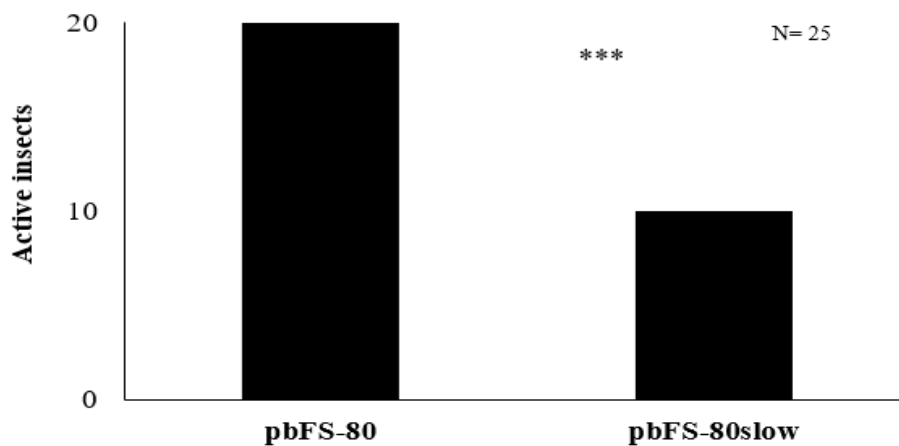


Figure 13 – Pulse repetition time. Number of individuals activated during the emission of the standard (fast) pbFS-80 and its slowed version (pbFS-80slow). The asterisks indicate significant difference ($p < 0.001$, Chi squared test, with Yates' correction).

In this case, the positional effect was not significant, although insects stimulated with pbFS-80slow showed an increased tendency to search after they previously experienced pbFS-80 ($p = 0.089$) (Figure 14). Perhaps testing a higher number of insects could have showed a significant difference between the two playbacks.

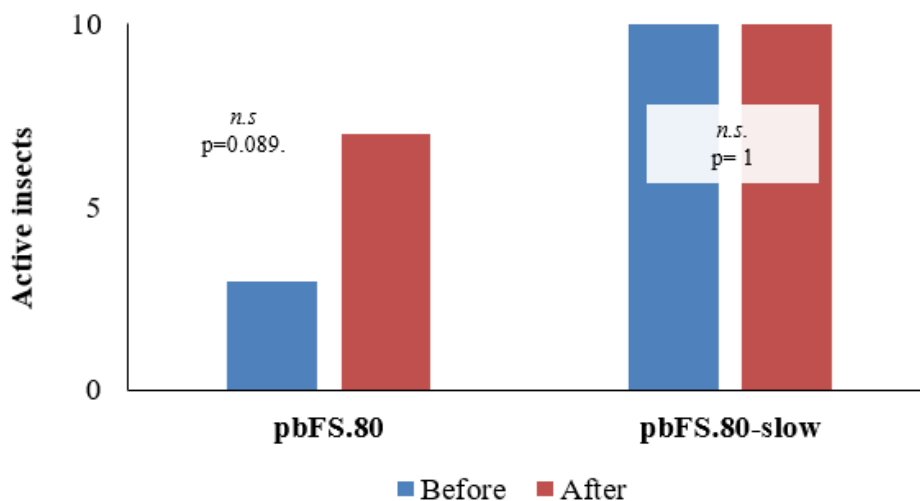


Figure 14 - Position effect of the playback, test 2. Number of insects active before and after the two playbacks. The emission of pbFS-80slow does not affect the subsequent response of the males, however pbFS-80 (fast) tends to increase the number of males responding to the following pbFS-80slow ($p=0.089$).

3. Wooden T-shaped arena - Test 3: “one choice test”

In test 3, 63 insects were released, 43% of which were behaviourally active (N=27). Among the active insects, a significantly higher number (Chi squared test: $\chi^2 = 21.9$, $df = 2$, $p < 0.001$) of males (N=21, 78 %) reached the shaking point and started loitering around the shaker, while 15% (N=4) did not reach it and 7% (N=2) reached the shaker that was turned off without loitering around it (Figure 15).

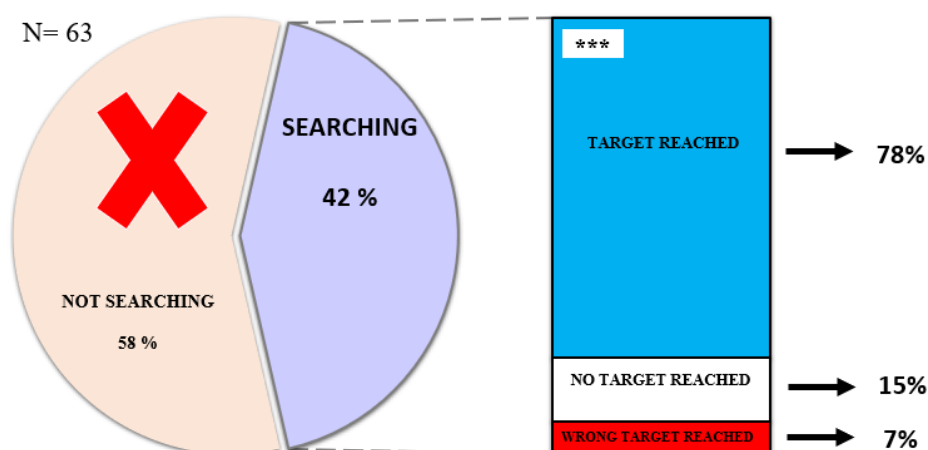


Figure 15 - Results of the single-choice test. The pie-chart shows the percentage of insects which did not search when the signal was broadcasted (red colour) and the number of insects which searched when the signal was broadcasted (blue colour). The column on the right refers on the insects whose search was triggered by the signal and reports their final choice. Target reached refers to the shaking arm, wrong target refers to the arm where the shaker was off, no target reached meant that no choice was done within the time given.

On average it took $3:01 \pm 01:01$ minutes for the individuals to reach the shaker.

4. Test 4, “two choices test”

In test 4, 40 insects were released of which 58% were active (N=23). Among the active insects, most of them (Chi squared test: $\chi^2 = 13.3$, $df = 2$, $p <$

0.001) reached the shaker that was playing *pbFS80* (N=16, 70%), 4% reached the shaker playing *pbFS160* (N=1) and 26% did not reach any target within the given time (N=6) (Figure 16).

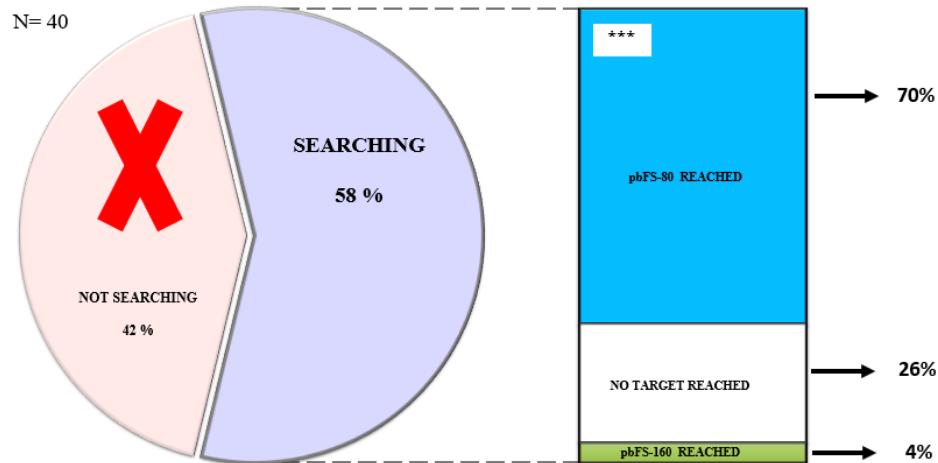


Figure 16 – Results of the double-choice test. The pie-chart shows the percentage of insects which did not search when the signal was broadcasted (red colour) and the number of insects which searched when the signal was broadcasted (blue colour). The column on the right refers on the insects whose search was triggered by the signal and reports their final choice. The column reports the percentage of insects which chose signal *pbFS-80* or *pbFS-160* and also the insects which did not take a choice in the time given.

On average it took $4:44 \pm 2:03$ minutes for the individuals to reach the shaker.

As the study conducted by Mazzoni *et al.*, (2017), the studies above confirmed the possibility to manipulate male's behaviour of BMSB using a mini-shaker to broadcast a female signal on artificial substrates.

The tests 1 and 2, showed that the original call recorded by Polajnar *et al.*, (2016) *pbFS-80*, performed significantly better than edited signals at activating males of BMSB. According to the results of test 1, the edited calls *pbFS-160* and *pbFS-even* activated less males compared to *pbFS-80*. Moreover, *pbFS-80* performed better at activating males while it was played, and it also elicited searching behaviour afterwards (see position effect). The even amplitude of the two harmonics in signal *pbFS-even* negatively influenced the performance of the signal and inhibited search for the next playbacks.

The analysis of the position effect on test 2, showed no significant difference between pbFS-80 and pbFS-80slow. However, after pbFS-80 was played, a tendency of more active insects could be observed ($p=0,089$), perhaps, testing a higher number of insects could have shown significant differences.

As a whole, test 1 and 2 demonstrate the importance of signal characteristics such as: the amplitude of the first and second harmonics and the pulse repetition time.

Test 3 and 4, confirm the capacity of active males to locate the artificially broadcasted female call. In addition to this, test 4 showed that active males of BMSB are able distinguish between two signals played simultaneously and reach the shaker broadcasting the best performing playback. The behaviour observed during the search was the typical walk and stop described in the previous chapter, in general the insects took a set of different choices before reaching the shaking spot. The number of right and wrong choices influenced the time required to reach the shaker and varied largely depending on the insect tested.

In the single choice test, the percentage of insects that reached the shaker (78%) was slightly higher than in the double choice test (70%). The percentage of insects who did not reach any of the shakers in the time given (see protocols), was higher in double choice test (26%) than in single choice test (15%). Also, the time required to reach the shaking spot was higher in double choice test (4:44 min, \pm 2:03) than in single choice test (3:01 min, \pm 01:01). From a direct observation, the search in the double choice test appeared to be less oriented.

Another interesting observation was that in the single choice test, the insects that chose the “vibration free” shaking arm, hid in shelters such as the holes in the prong clamp that was holding the shaker. In this case, the behaviour observed appeared as if males stopped being interested in searching for the signal and thus looked for some place to stay.

In test 4, only 4% ($N=1$) of the insects loitered around the shaker emitting signal pbFS-160. In this case, two hypotheses were raised, one is that some males were actually more attracted by signal pbFS-160, the other is that their searching behaviour was triggered by pbFS-80 but they failed to localize the source of the vibration.

The percentage of active males varied greatly among the 4 tests, ranging from 80% (test 2) to 19% (test 1) (average = 43%). Such great variation was hard to explain and could perhaps be due to several factors such as: age of

the insect, weather, photoperiod and so on. Since the exact age of the insects used was unknown, possibly some of them were too old or tired from the overwintering period. The patterns of atmospheric pressure and weather were observed but there did not seem to be a direct link between weather and willingness to mate.

Test 4 showed that the wooden T-shaped arena developed can be a valuable tool to study different signals in their performances to attract males of BMSB. On the wooden-arena, insects were able to distinguish simultaneously broadcasted signals. This tool could be used for future research in order to test and compare new recorded signals to refine and improve attractiveness. New prototypes could be developed with the goal to test new materials or wood thickness.

2. Stakeholder analysis

1. Findings

The respondent group was characterized by a high heterogeneity of professional skills and goals. For most of the participants, the BMSB was perceived as a major threat to agriculture.

Some of the respondents explained that, now, the problem is present in limited areas, mainly in the southern part of the region. In the last growing season, late outbreaks have been reported in the northern part as well, but mainly on late ripening apple varieties such as Fuji. This indicates that the population of the pest is still expanding in this area. The potential to damage crops largely depends on population growth, which is difficult to predict. In the colonized areas the population grows very quickly and when bugs are high in number, they become visible on ripening fruits. So far BMSB outbreaks are observed mainly before and during the harvest.

The interview respondents reported that the core problem is the lack of natural enemies.

“If you don’t have something natural that controls the population, insects become scary! (...) I tried to train our team of advisors and also farmers. Knowledge exchange is very important.”

(Farmer advisor, 2019)

“It is a pest, a sort of tank that no one out there wants to eat!”

(Manager 2, 2019)

As reported by the researcher, the areas where BMSB is already established experienced a very quick crash of IPM practices due to the increased insecticides application.

“BMSB is one of the most invasive insects we have had in the last years. (...) It has caused a negative revolution of IPM and we have lost ground in positive agricultural practices. IPM practices are not working against the BMSB, it will have a huge impact!”

(Researcher, 2019)

Interestingly, when growers were interviewed, they appeared surprised of taking part in a research and that their knowledge was considered relevant. Growers often asked questions about the biology and current strategies to control BMSB and often mentioned their lack of knowledge about what is going to happen and asked how to overcome the challenge of BMSB.

“From the information I have, I know it is very difficult to monitor BMSB due to its phytophagy but I know very little about the biology. To become aware of the problem, farmers need to experience the problem. We know that the insect has now been here for 2 years, but we still have to learn a lot!”

(Apple grower 3, 2019)

Given this scenario, the local DFA is working and supporting research activities on different control strategies that include: biotremology, biological and chemical control. The local DFA appeared to have a clear orientation. Among the possible control strategies, biological control is perceived as the most promising.

“We are waiting for an agreement with the national departments of agriculture and environment. I asked the department if FEM could become an active partner in the experimentation phase needed for the approval of the release of the allochthonous insect (...) They’ll have to approve a decree with some guidelines. Clearly experimentation must be done, you cannot release

the wasp directly, without thinking about possible consequences. (...) If we could manage to introduce something that controls the problem, all would benefit from it."

(Councillor for agriculture, 2019)

In general, the biological control was perceived also by the other respondents as the most pragmatic solutions. However, growers and managers complained about the time required by institutions to act.

"Solutions to mitigate the problem are now researched, but we have been too slow! (...) Political approach has limits, it strives to find slogans and not long-term strategies."

(Manager 3, 2019)

"European laws to introduce antagonists are too strict. (...) It is hard to let growers know that we are not just waiting for a solution, but actively searching for it".

(Manager 1, 2019)

Some growers for example argued that since the balance of endemic species is already altered by invasive species such as BMSB, introducing a new species doesn't represent a problem.

"BMSB is an alien species and therefore also the parasitic wasp can be introduced. The parasitic wasp will come, in a way or another!"

(Apple grower 1, 2019)

Meanwhile, insecticide-based control was considered of low efficacy. For growers, this was mostly evidence reported by the experiences of colleagues in other regions. However, a farmer reported that some phytosanitary product distributors are spreading the voice that some banned pesticides could keep population under control.

OPs managers reported that this strategy might have very important implications. Increasing the number of insecticides applications, both in terms of quantity and type of active ingredient used, increases the chemicals residue

on fruits. On European markets, many retailer companies strive to minimize chemical residues in the fruits. For this reason, the chemical control strategies adopted to control BMSB could negatively affect the marketability of fruits, with obvious implications for the profitability of farms.

“If I was a buyer, I would buy apples somewhere else”

(Manager 2, 2019)

Despite the current increased use of insecticides, the respondent researcher, the farmer advisor and the scientific literature (reported in the introduction) warn that chemical control has a low efficacy in keeping BMSB under damage thresholds. When interviewees were asked about their knowledge on vibrational communication, most of them appeared to know very little about that. However, after a general explanation, the issue raised about this experimental technique was regarding its economic and technical feasibility.

So far, the only effective solution seems to be the use of insect nets that mechanically screen the orchards from BMSB. This strategy is becoming widespread in Emilia Romagna, Veneto and Friuli. However, the interviews carried out in Trentino, reveal that this practice might not be viable on most of the local apple farms. The explanation why it is not considered viable, largely depends on the personal attitudes.

Some growers raised issues of identity and aesthetic of landscape.

“The use of nets has several advantages, it even protects from the hail but for the landscape it is a monstrosity. Using insect nets makes farming as living in a metropolis!”

(Apple grower 4, 2019)

Clearly, considering the need to commercialize the apples, if crops are spoiled by BMSB and insecticides applications is not a viable solution, growers cannot afford reject plastic due to aesthetic parameters.

“Growers don’t like plastic, and then, the impact on the landscape! Sometimes I look at mountainous landscapes and wonder what tourists would think. Personally, I am strict with plastic abuse, but growers cannot afford to critique plastic.”

(Farmer advisor, 2019)

Another reason why insect nets are not viable is orchards fragmentation. As explained by growers, most of the orchards are small, have irregular geometries and are not even designed to be covered by insect nets. In order to cover orchards with insect nets, they should be re-planted.

“Due to fragmentation and people mindset, it is impossible to think about covering all the plots with nets.”

(Apple grower 3, 2019)

Managers and the councillor for agriculture considered insect nets as local solutions in areas that are particularly problematic. However, they cannot be considered the solution to the problem. There is in fact another source of incompatibility related to insect nets and the apple production in Trentino. Some respondents speculated about the scenario in which apple orchards were all screened against BMSB and they argued that this would have a negative economic side-effect for tourism.

“This issue could become social rather than specific of the agricultural sector. Somehow this emergency helps society to understand that agriculture is part of the society. (...) If agriculture doesn’t find solutions, this could interfere with 95% of the population. An agricultural crisis could potentially interfere with other pillars of the income generated in some areas of the region.”

(Manager 1, 2019)

This last interview citation showcases that agriculture is an open system. Projections of “worst-case scenarios” related to BMSB indicate that this pest can put in trouble the sustainability of wide areas, due to its direct impact on human activities that goes beyond farming.

In order to summarize the patterns that were identified in the interview transcripts, three main themes were identified: control strategies, knowledge and product marketability. The scheme proposed in figure 15 represents

the connection between the themes identified, the different stakeholders and the main issues related to the control strategies.

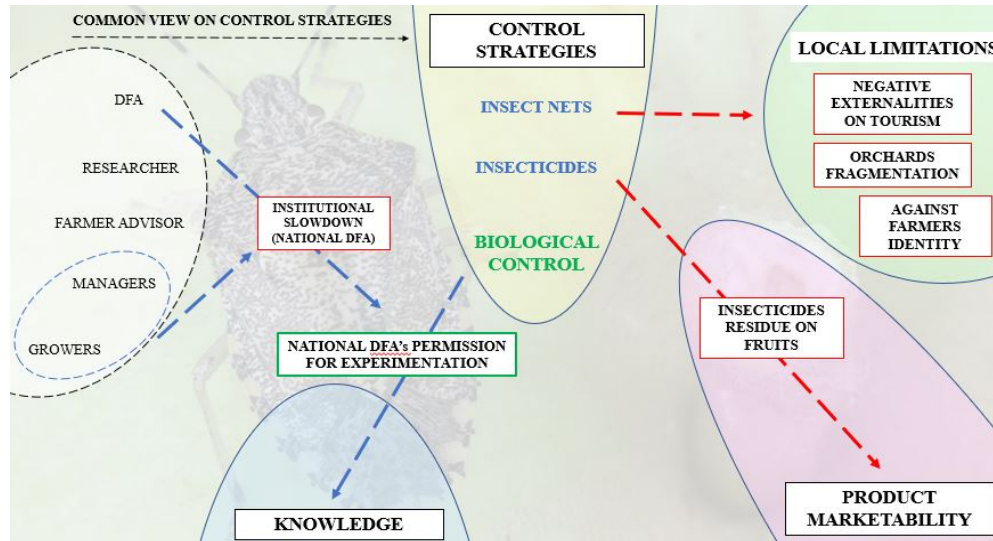


Figure 15 - Graphic summary of the themes identified in the TA and the related issues perceived by SSI respondents. The boxes outlined with black indicate the main themes: knowledge, control strategies and product marketability. Blue control strategies were considered not feasible whereas green colour indicates that the strategy is promising. The boxes outlined with red indicate issues related to each control strategy. The box outlined with green represent a step required to test the feasibility of the control strategy. Blue dashed arrows represent interaction among stakeholders and issues related to feasible control strategies. Red dashed arrows highlight conflicts related to a given control strategy.

In general, the perception of control strategies is rather shared among the respondents. Insect nets and insecticides application are not considered feasible and this is due to different reasons. Insect nets are not feasible due to local limitations, whereas insecticides are characterized by a relatively low efficacy and are challenging apples marketability.

The most surprising finding was the lack of political and institutional action from the national DFA towards the experimentation of the new parasitic insect. This fact was mainly raised by growers and managers. The first established populations of BMSB were found in Italy in 2012 (Maistrello *et al.*, 2016) and after that, in many regions damages on agricultural productions have been enormous (Bianchi, 2018). The dangerousness of BMSB was ascertained in the late 90's in North America. On the 12th of June 2019, the

Italian government put a preliminary plan for investigating *T. japonicus*. The release of alien species is forbidden by the Decree 92/43/CEE published on the 21st May 1992 and the only chance to derogate from such decree is to bring scientific evidence that proves the absence of negative side-effects of the species. Once the results are available, they have to be submitted to the Ministry of Agriculture and Environment (Drogo, 2019). The institutional process will require time before some research about *T. japonicus* will be available. Interestingly, in New Zealand the release of *T. japonicus* was approved in case of need and so far, no BMSB was found yet.

5. Discussion

Finding solutions to control plant-dwelling insects such as BMSB is of paramount importance in order to develop and maintain IPM strategies. Among the driving forces for this, is the increasing awareness of consumers about the negative impact of chemicals on the environment and human health (Polajnar *et al.*, 2016)(Maistrello *et al.*, 2016). This creates space for a new market that seeks for alternative tools to reduce chemical residues on agricultural products. Plant protection strategies that include vibrational signals are in line with this new trend.

So far, vibrational communication has not been exploited as pest control method in commercial farms, however, an experimental field trial has been conducted in northern Italy for the purpose of testing mating disruption in an experimental vineyard at FEM (Polajnar *et al.*, 2016). In this vineyard, the aim was to control *Scaphoideus titanus* (Homoptera : Cicadellidae), an important vector of the phytoplasma responsible for Flavescence doreé, a world-wide spread disease which control is mandatory and based on insecticides. For *S. titanus* occurs through vibrational signals and the mating communication occurs exclusively on grapevine where the insect carries out its entire life cycle (Mazzoni *et al.*, 2009). In the experimental vineyard, a set of shakers was used to emit vibrational signals to interfere and disrupt mating. In the trial, the wires and poles that constitute the trellis were used as media to transmit the vibration. The results suggested an effective mating reduction, however, the authors highlighted that the effect of the shakers in the vineyard worked well only in very limited field environment. The signal emitted by the shaker was dissipated close to the poles where the metallic wires of the trellis were touching the poles and thus amplitude of the disrupting signal was attenuated.

For the development of “vibrational plant protection strategies” it is important to have relatively high active areas, where shakers are still effective in manipulating the target insect’s behaviour. Moreover, the technology adopted to send such signals should be fine-tuned in order to have a low energetic demand, to avoid negative side effects on crops and to affect beneficial insects (Polajnar *et al.*, 2015).

The laboratory experiments on BMSB allowed to bring forth the understanding on the playback parameters that affect the “searching” behaviour of the males of BMSB. At this stage, it can be said that the female call pbFS-80 is not ready to be used for the purpose of mass trapping with “attract and kill” strategies. The population targeted in fact, is composed exclusively by adult males that are willing to mate. The experiments showed that the males reacting to the signal was variable, ranging from about 20 to 60 %. Despite these limitations, if adopted on a large scale by a high number of growers the decrease of males in the population could decrease mating rate and thus population. The attractivity of the signal pbFS-80 on active males, could be also used for “early detection” strategies in areas that are under risk of BMSB colonization (airports, stations, merchant ships). The core problem for the development of vibrational traps, is to create tools that have wider active areas so to reduce the number of traps needed per unit of surface. The synergistic effect of vibrational signals and aggregation pheromones still needs to be tested. Potentially it could increase the capture rates of possible “attract-and-kill” traps. Currently, the problem with pheromone baited traps is that BMSBs are attracted by them but low captures are achieved (Aldrich *et al.*, 2009), for this reason, they are not widely used to control BMSB.

Despite the relatively unexplored, but yet high potential of biotremology in providing “insecticide-free” control for BMSB and other insects, this discipline is still taking shape in the academy. Most likely, the actual lack of vibrational control strategies is the reason why it is almost unknown to those who are not researching in the field.

The biological control, instead, is generally perceived as a safer and more acceptable control technique compared to the traditional application of pesticides to control agricultural pests (McNeil *et al.*, 2010). The stakeholders’ view about the biological control that *T. japonicus* could provide, suggests shifting the research focus mainly in this direction. It is important to highlight that the introduction of alien species can lead to severe and unexpected damages to agriculture and more in general to wildlife (Sheail, 2003). With regard to BMSB, some host-range tests conducted in the US showed the greater potential for parasitoid development in BMSB eggs compared to

other non-target species (Hedstrom *et al.*, 2017). The results obtained in the US are promising, however, similar researches need to be carried out on the endemic species present in Italy. This step is essential to assess the feasibility of inundative release of the control agent, however, since no permission was yet given by the national DFA, no effective biological control can be expected for the next growing seasons.

The areas where BMSB was first found in Italy, experienced a fast population growth which quickly led to severe economic damages on crops within few years after the first detection (Costi *et al.*, 2017). In Trentino, colonization is at a relatively early stage, but the pest is quickly spreading in most growing areas. A moderate control of BMSB can be expected from the native egg parasitoids such as *A. bifasciatus* (Stahl *et al.*, 2019) but some research is needed in order to maintain the achievements obtained with IPM strategies in the last decades (Ioriatti *et al.*, 2011).

The social survey allowed to bring forth the understanding of the phenomena in the region of Trentino collecting different stakeholder's perceptions in a systematic way. Clearly, the results were obtained on a small sample of interviewees and in a relatively "small area". Because of this, results and perceptions were partly influenced by local factors. In the next years, local and national policy makers will be required to take decisions on the topic; the results reported in this work could support such choices.

As a general consideration, bottom-up approaches to support the decision-making process and knowledge creation are not common in the area. Growers and managers complained about the lack of support by the department of agriculture, especially with regards to the introduction of *T. japonicus*. As final suggestion, in order to improve the interaction between stakeholders, the development of Participatory Action Research (PAR) initiatives should be introduced as a default practice. This approach to research seeks full collaboration from participants in order to analyse data and "take action". A widespread method to conduct PAR is the "focus group", which is composed by individuals who have relevant characteristics for the topic of the study. In the case of the apple industry in Trentino, the participants could be, for example, representatives of the institutions already included in the SSI. PAR is often used as action-research method in agriculture (Macdonald, 2012) and its principles perfectly overlaps with agroecological values (Méndez *et al.*, 2013).

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7. Interview guides

1. Growers

1. When have you first heard talking about BMSB?
 - a. Can you distinguish it from other endemic pentatomid species?
 - b. Do you know its biological cycle?
2. Did you find BMSB in your farm?
 - a. Did it damage harvest?
3. Do you consider it a dangerous species?
 - a. If so, why?
4. Do you consider monitoring important?
5. What is in your opinion, a key tool to control this BMSB?
6. Have you ever heard about vibrational communication among insects?
7. Are there institutions from which you expect answers to control BMSB?

2. Managers

1. When have you first heard talking about BMSB?
2. Do you think this species could be dangerous for Trentino's apple industry?
3. Do you talk about BMSB with producers?

4. Have you observed damages during the storage phase of apples so far?
5. Do you consider monitoring important?
6. What is in your opinion, a key tool to control this BMSB?
7. Have you ever heard about vibrational communication among insects?
8. Would your organization finance research projects on BMSB?
9. Are there institutions from which you expect answers to control BMSB?

3. Researcher / advisor

1. Do you think this species could be dangerous for Trentino's apple industry?
2. What are the aspects that characterize this species?
3. Is Trentino agroecosystem susceptible to this pest?
4. What is in your opinion, a key tool to control this BMSB?
5. Are there institutions from which you expect answers to control BMSB?

4. Councillor for agriculture

1. What is the Trento province doing to tackle BMSB issue?

2. What is considered the key tool to control BMSB?
3. What are the steps needed to introduce the parasitoid?
4. Are you also financing alternative control strategies?
5. Is vibrational communication considered a possible tool as well?